REPRODUCTIVE INDICES OF MALE SNOW CRAB, *CHIONOECETES OPILIO*, FROM THE EASTERN BERING SEA

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August 10, 2012
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A

THESIS

Presented to the Faculty

of the University of Alaska Fairbanks

in Partial Fulfillment of the Requirements

for the Degree of

MASTER OF SCIENCE

By

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Fairbanks, Alaska

August, 2012

Abstract

The reproductive success of male snow crab (*Chionoecetes opilio*) is complicated by two different life history stages; male *C. opilio* undergo a terminal molt prior to adulthood which is marked by an allometric change in chela size. While adolescent males can produce spermatophores, terminally molted males are most successful in mating and reproduction. Molting and mating are hormonally linked, with molting regulated primarily by ecdysteroids and mating regulated by the putative reproductive hormone methyl farnesoate (MF). Methyl farnesoate is structurally related to the insect juvenile III hormone and, in addition to reproduction, may have a role as a juvenilizing hormone in crustaceans. The purpose of this study was to determine how molting affects the reproductive biology of snow crab by comparing the gonadosomatic index(GSI) and concentrations of circulating MF in adolescent and adult males. I used shell condition as a rough estimate of time post molt to compare GSI and MF between new-shell males, those that had molted within one year, and old-shell males, those that had not molted for at least 1 year. I measured GSI as the ratio of gonad weight to whole crab weight and used GSI as a proxy for reproductive fitness; I quantified circulating MF using high performance liquid chromatography. New-shell adolescent and adult males had significantly lower GSI than old-shell males; thus molting compromises the reproductive physiology of male *C. opilio*. New-shell adolescent males had significantly higher MF levels than old-shell adolescent males, and MF levels remained low after the terminal molt, supporting a juvenilizing role for MF in *C. opilio*.

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Acknowledgements

I would like to thank my advisor Dr. Sherry Tamone for all her guidance, friendship, and patience. I also thank Dr. Ginny Eckert and Dr. Gordon Kruse for their countless edits and insights throughout this project.

I am indebted to Alaska Department of Fish and Game staff for all their experience and help during crab collections, especially Laura Stichert, Jan Haaga, and Doug Pengilly. I am thankful for the assistance of my fellow Crab Lab students Joel Webb, Miranda Westphal, Kevin McNeel, Melissa Rhodes-Reece, Dr. Jodi Pirtle, and Courtney Lyons. I would also like to thank my fellow students for their support throughout the semesters and for sitting through practice talks, including Janelle Mueller, Suzie Teerlink, and Gretchen Geiger.

This project was funded through the North Pacific Research Board (Project 812). I also thank the University of Alaska Fairbanks and the University of Alaska Southeast for teaching assistantships. I would like to further thank the University of Alaska Southeast and the Auke Bay Lab for the facilities to house our snow crabs, and the Atkinson lab for providing space for the HPLC. I am thankful for all the help and encouragement from UAF and UAS faculty and staff, especially Gabrielle Hazelton, Louisa Hayes, Jolene Troupe, Debi Rathbone, and Lawrence Powers.

Finally I would like to thank my husband Adam Zaleski for his unwavering love and support throughout the years, giving advice when needed and learning more about crabs than he ever wanted.

General Introduction

Snow crab *Chionoecetes opilio* (O. Fabricius, 1788) are commercially harvested in the North Pacific and Atlantic Oceans in male-only fisheries. Newly molted males make up the majority of commercial catches because they are targeted for their visual appeal over males with older, darker shells marked by abrasions and epibiotic growth (Conan and Comeau, 1986). Management of the eastern Bering Sea (EBS) stock includes a size limitation to ensure that each male has at least one opportunity to mate before harvest (NPFMC, 1998). This assumption may not be satisfied, because recent molting may compromise the reproductive potential of some crabs that are commercially targeted.

A. Eastern Bering Sea *C. opilio* Fishery

Chionoecetes opilio represents one of the most commercially valuable crab fisheries in the EBS. These crab inhabit cold water where near bottom temperatures range from -2.0 to 4.0°C (Otto, 1998). As both male and female *C. opilio* mature, they move offshore and southward toward the middle domain of the EBS (Orensanz et al., 2004). A recent shift in the distribution of mature males and females is concurrent with the northward retreat of the extent of the cold pool (< 2°C) on the EBS shelf (Orensanz et al., 2004; Zheng and Kruse, 2006). The shift of mature female distribution northwest is hypothesized to reduce the recruitment strength for the southern portion of their range (Zheng and Kruse, 2006). Historical recruitment to the EBS *C. opilio* stock varied substantially through time but has generally been low in the past decade (Orensanz et al., 2004; Zheng and Kruse, 2006; NPFMC, 2011).

Commercial crab populations in Alaska including red king crab (*Paralithodes camtschaticus*), blue king crab (*P. platypus*), Tanner crab (*C. bairdi*), and *C. opilio*, decreased between the 1980s and 1990s with little sign of recovery (Zheng and Kruse, 2003; Woodby et al., 2005). The EBS *C. opilio* stock continued to decrease and collapsed in 1994 (Otto, 1998), though strong recruitment was observed after the collapse with a secondary peak in harvest in 1998 (NPFMC, 2008). Spawning stock biomass fell below the minimum stock threshold in 1999 (Kruse et al., 2007). The fishery was declared overfished by the National Marine Fisheries Service (NMFS) in 1999 (Zheng et al., 2002). Before the collapse, the landing of commercial-sized males peaked in 1991 at 328.6 million pounds (Bowers et al., 2008).

Presently, the *C. opilio* fishery is a size restricted, male-only fishery with a majority of crab retained having a carapace width (CW) greater than 101 mm (NPFMC, 2008), though the minimum size limit is 78 mm CW (Bowers et al., 2008). The guideline harvest level for male *C. opilio* prior to 2000 was 58% of males with a CW >101 mm, estimated from trawl surveys performed in the EBS by NMFS (NPFMC, 2008). A new harvest strategy for rebuilding the stock within 10 years, as required by the Magnuson-Stevens Fishery Conservation and Management Act (NMFS, 1996), was implemented in 2000 using a harvest rate of 20% of estimated males with CW >101 mm (NPFMC, 2008). The rebuilding period surpassed the 10 year goal; the stock was declared rebuilt in 2011 with EBS stock estimates having been greater than B_{MSY}, set at 921.6 million pounds, for two consecutive years (NPFMC, 2011), as required by NMFS (NPFMC, 2000). Current

management of the fishery uses an assessment model separating crab by sex, shell condition, and maturity (NPFMC, 2011).

B. Terminal Molt

Adult *C. opilio* are anecdysial and will no longer grow after the terminal molt (Conan and Comeau, 1986). The terminal molt to maturity in female *C. opilio* is well accepted (Watson, 1972). Though there had been much debate regarding the existence of a terminal molt for male *Chionoecetes* crab (Conan et al., 1988; Donaldson and Johnson, 1988; Elner and Beninger, 1989), it has now been established both in laboratory and field studies (Conan and Comeau, 1986; Sainte-Marie and Hazel, 1992; Otto, 1998; Tamone et al., 2005; Tamone et al., 2007) and is considered an important distinction in crab biology for management (Rugolo et al., 2005; NPFMC, 2011).

Although male *C. opilio* molt several times over their lifespan, they fully mature after the second of two critical molts (Comeau and Conan, 1992). Immature males undergo the first critical molt, the juvenile molt, after which they begin sperm production and are considered adolescents. The second critical molt is the terminal molt which is distinguished by an allometric increase in chelae height compared to CW (Conan and Comeau, 1986; Sainte-Marie and Hazel, 1992). The differentiation that occurs during this terminal molt is likely regulated by hormones (Laufer et al., 2005; Tamone et al., 2005). Because the molt to maturity is based on a conspicuous change in chelae height, and not on a specific carapace width, there is high variability in age and size for male *C. opilio* at the terminal molt (Sainte-Marie et al., 1995; Comeau et al., 1998) which may result from

changes in male-to-female sex ratios or environmental variations (Sainte-Marie et al., 2008).

C. Snow Crab Reproduction

Female *C. opilio* have a terminal molt, after which they become reproductively mature (Watson, 1972). Prior to the terminal molt, a female is considered pubescent; following the terminal molt, but before oviposition, a female is called nulliparous (Sainte-Marie et al., 1999). A terminally molted female with her first clutch is defined as primiparous and a female with subsequent clutches is defined as multiparous (Elner and Beninger, 1992). Females have spermathecae for spermatophore storage (Elner and Beninger, 1992; Sainte-Marie and Sainte-Marie, 1998), thus females can use stored sperm in the absence of males for new clutches (Elner and Beninger, 1995).

Several types of mating pairs may occur in wild *C. opilio* populations. These pairs are contingent upon the size of male chela, signifying adolescence or adulthood, and the timing post-molt and number of previous clutches carried by the female. Adolescent or small adult males may copulate with nulliparous females or attempt to mate with primiparous females, and large adult males copulate primarily with multiparous females (Sainte-Marie and Hazel, 1992; Moriyasu and Comeau, 1996; Sainte-Marie et al., 1999; Kruse et al., 2007). Adult males represent a greater percentage of males observed in wild mating pairs compared to adolescent males (Sainte-Marie and Hazel, 1992; Claxton et al., 1994), so the probability that adolescent males will have access to reproduce with females may depend on the presence or absence of adult males. Larger claws give adult

male crabs a competitive advantage through stronger grasping ability (Stevens et al., 1993; Sagi et al., 1994; Sainte-Marie et al., 1997; Oliveira and Custódio, 1998); the allometric change in chela size is considered a secondary sexual characteristic (Comeau and Conan, 1992).

D. Molting and Reproductive Hormones

Molting and reproduction are regulated by hormones that are synthesized by specific endocrine glands and secreted into the hemolymph. The X-organ sinus gland complex is a neuroendocrine gland in decapod crustacean eyestalks (Pyle, 1943) which regulates physiological processes, including reproduction and growth, through the release of inhibitory neuropeptides (Chang et al., 1993; Reddy and Ramamurthi, 1999). Reproduction is indirectly regulated by the mandibular organ inhibiting hormone, the removal of which promotes gonad maturation (Laufer et al., 1987b; Rotllant et al., 2000). Molting is regulated by molt-inhibiting hormone which, when removed, promotes an increase in ecdysteroid secretion (Chang et al., 1987; Chang et al., 1993; Tamone et al., 2005) and the initiation of molting physiology.

Reduced levels of circulating ecdysteroids are characteristic of terminally-molted male *Chionoecetes* crab (Tamone et al., 2005; Tamone et al., 2007). Recently molted juvenile *C. opilio*, characterized using dry chela weight rather than chela height, had higher hemolymph ecdysone than mature males of a similar shell condition (Cormier et al., 1992). The decrease in ecdysteroid synthesis and secretion after terminal molt is attributed to the anecdysial physiology of mature males, who exhibit subsequent

degeneration of the Y-organ and over-production of molt-inhibiting hormone from the X-organ sinus gland (Carlisle, 1957).

Methyl farnesoate (MF) is a sesquiterpenoid hormone produced by the crustacean mandibular organ (Borst et al., 1987) and is structurally related to the insect juvenile hormone (Laufer et al., 1987a). A sinus gland extract, mandibular organ inhibiting hormone, inhibits mandibular organ activity (Laufer et al., 1987b) and maintains the inhibitory regulation of MF (Liu and Laufer, 1996; Chaves, 2001). Binding proteins specific for MF were identified in hemolymph of *C. magister* (Tamone et al., 1997) and target tissues for MF include reproductive tissues (Laufer et al., 1996), suggesting a role in reproduction.

Methyl farnesoate regulates reproductive biology including gonad maturation and behavior. Exogenous MF stimulates gonad development in freshwater prawn *Macrobrachium malcolmsonii* (Nagaraju et al., 2003), green crab *Carcinus maenas* (Nagaraju and Borst, 2008), Indian field crab *Oziotelphusa senex senex* (Kalavathy et al., 1999), and *L. emarginata* (Sagi et al., 1993; Sagi et al., 1994). Mating behavior is correlated with increased MF concentrations in male crustaceans; the highest levels of MF were measured in the most reproductively active *L. emarginata* (Sagi et al., 1994; Laufer and Ahl, 1995) and *C. bairdi* (Laufer et al., 1996). A disparity of MF levels between non-reproductive and reproductive phases was observed in crayfish *Procambarus clarkii* (Laufer et al., 2005) and *C. maenas* (Nagaraju and Borst, 2008), where reproductive phase males had greater MF than males in non-reproductive phases.

Another potential role for MF in crustacean physiology is the regulation of development. Elevated concentrations of MF may inhibit development to maturity in juvenile and adolescent crustaceans. Increased MF, induced by eyestalk ablation in adolescent male *L. emarginata*, resulted in no allometric change in claw size after molting (Laufer et al., 2002). Control animals did not experience an increase in MF prior to molting and proceeded with a molt to morphometric maturity with larger claw differentiation (Laufer et al., 2002). The inhibition of maturation due to the presence of MF in juvenile animals suggests that low levels of MF may be measured in old-shell adolescent male *C. opilio* approaching their terminal molt.

E. Shell Condition

The shell condition of a crustacean is a rough estimate of the amount of time that has passed since the last molt. Older shells by appearance represent crab with longer post-molt periods (Fonseca et al., 2008). New-shell male *C. opilio* are considered crab that have molted within the last year, while old-shell crab have not molted for the past year or more (Nevissi et al., 1996). Mating behavior in *C. bairdi* and *L. emarginata* is associated with shell hardness, or increased time post-molt (Paul et al., 1995; Laufer et al., 1996). In both *C. bairdi* and *L. emarginata*, lower levels of MF were measured in new-shell males compared to old-shell males (Laufer et al., 1996), thus shell condition can successfully be used to compare physiological processes during and after a molting period.

New-shell male *C. opilio* are more commercially valuable for their visual appeal (Conan and Comeau, 1986). As a preferred target for the fishery, new-shell adult males are overrepresented in the harvest when compared to their proportions in the population (Conan and Comeau, 1986), but they may be harvested before adequate time has elapsed for rebuilding reproductive tissues and thus may not be reproductively successful before removal from the population. Additionally, some proportion of harvested new-shell males may have lower levels of reproductive hormones and may not be reproductively active prior to harvest in the commercial fishery (Sainte-Marie et al., 1995; Moriyasu and Comeau, 1996; Fonseca et al., 2008).

F. Purpose

Male physiology is often overlooked when studying *C. opilio* reproduction. It is assumed that commercially harvested adult males have successfully mated at least once before entering the fishery (NPFMC, 1998). If reproductive hormones and gonadal structures are minimized during and directly after the terminal molt, it is possible that new-shell adult males are not reproductively contributing to the population. Removing larger males gives smaller adult males more opportunities to mate. Greater frequency of smaller adult males selects for smaller males in the population by increasing their mating efficiency (Comeau and Conan, 1992; Abbe, 2002; Carver et al., 2005; Sato et al., 2005). The primary objectives of this project were to examine the effects of molting on the reproductive physiology of male *C. opilio*.

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Chapter 1: RELATIONSHIP OF MOLTING, GONADOSOMATIC INDEX, AND METHYL FARNESOATE IN MALE SNOW CRAB (*CHIONOECETES OPILIO*) FROM THE EASTERN BERING SEA[1]

[1] Marilyn F. Zaleski and Sherry L. Tamone. Journal of Crustacean Biology. To be submitted.

ABSTRACT

Snow crab, *Chionoecetes opilio*, have a complex mating system, and understanding their reproductive patterns is paramount to crab fishery management. Mating and molting are inextricably linked, so the energetically expensive molting process may compromise male mating potential for some period after the molt. Recently molted, new-shell males are targeted by the commercially valuable eastern Bering Sea (EBS) fishery. Old-shell males, or males that have not molted within the last year or longer, are not targeted to the extent of new-shell males, though they may have had enough time to build up their reproductive tissues after molting and before the fishery occurs. We examined a crustacean reproductive hormone, methyl farnesoate (MF), and reproductive fitness, using gonadosomatic index (GSI) as a proxy, in EBS male snow crab to determine any differences in reproductive potential between adolescents and adults. We compared these reproductive indices in new- and old-shell males as a broad estimate of the effect of molting biology on reproduction. Circulating MF was significantly higher in new-shell adolescent males compared to old-shell adolescent males and old-shell adult males, suggesting a juvenilizing rather than gonadotropic role for MF in *C. opilio*. New-shell males had a significantly lower GSI compared to old-shell males for both adolescent and adult males. The lower GSI measured in new-shell adults compared to the significantly higher GSI levels measured in old-shell adults suggests that new-shell males harvested during the commercial fishery are not as reproductively fit and therefore may not be contributing to the population.

INTRODUCTION

Snow crab, *Chionoecetes opilio* (O. Fabricius, 1788), is a commercially important species in the North Pacific and North Atlantic Oceans. A clear understanding of their growth and reproductive biology is important for the management of fisheries for this species. The eastern Bering Sea (EBS) fishery is managed by seasons and restricted to male-only with a minimum size limit of 78 mm carapace width (CW) that attempts to ensure each harvested male is reproductively mature (NPFMC, 2008). Harvest of *C. opilio* peaked in 1991 with a landing of 149,050 metric tons (Figure 1) (Bowers et al., 2008); however, the stock collapsed and was declared overfished by the National Marine Fisheries Service (NMFS) in 1999 (Zheng et al., 2002). A new management strategy was adopted in 2000 and the fishery reached a rebuilt status in 2011 (NPFMC, 2011).

Classifying different shell conditions of crustaceans, using carapace markings, deterioration, and epibiotic growth, can be used as a rough estimate of time elapsed since molting (Fonseca et al., 2008). The EBS *C. opilio* fishery targets "new-shell" male *C. opilio* that have hardened, unabraded carapaces (Bowers et al., 2008). The market preference for new-shell males is due to their clean appearance and is reflected in their superior commercial value (Conan and Comeau, 1986). "Old-shell" males have not molted for over a year and as such have highly abraded carapaces and potential epibiotic growth that scales with time since the last molt.

Chionoecetes opilio males undergo two important maturation molts during their life cycle (Comeau and Conan, 1992). The first important molt is the juvenile molt, after which males are considered "adolescents" and begin to produce functional

spermatophores (Comeau and Conan, 1992; Sainte-Marie et al., 2008). As adolescents, *C. opilio* continue to molt and grow proportionately to their previous size until the terminal molt, signified by an allometric change in chela size (Conan and Comeau, 1986; Comeau and Conan, 1992) and a significant decrease in circulating molting hormones (Tamone et al., 2005). The allometric change in chela size is important for grasping potential mates and competing for females with other males (Comeau and Conan, 1992). The terminal molt is also known as the molt to morphometric maturity (Conan and Comeau, 1986; Otto, 1998). Here, we refer to terminally molted males as "adults" and males producing spermatophores but still awaiting the terminal molt as "adolescents" (Beninger et al., 1988; Sainte-Marie and Hazel, 1992; Sainte-Marie et al., 1995).

Crustacean growth and reproduction are under endocrine control, in part, by ecdysteroids and methyl farnesoate (Chang, 1993). Ecdysteroids are synthesized by the crustacean y-organs and promote multiple aspects of molting physiology (Chang et al., 1976; Chang and O'Connor, 1977; Keller and Schmid, 1979). Methyl farnesoate (MF) is a crustacean reproductive hormone (Laufer and Biggers, 2001), which is synthesized and secreted by the mandibular organ (MO) (Borst et al., 1987) and was first characterized in the spider crab *Libinia emarginata* (Laufer et al., 1987a). It has since been identified in over 30 crustacean species (Table 1). Secretion of MF from the crustacean MO is regulated through MO inhibiting hormones produced by the eyestalk X-organ sinus gland. Eyestalk ablation resulted in an increase of circulating MF in the Norway lobster *Nephrops norvegicus* (Rotllant et al., 2001), *L. emarginata* (Laufer et al., 1987b; Laufer et al., 2002), and the American lobster *Homarus americanus* (Li et al., 2010). After

secretion from the MO, MF circulates through hemolymph bound to MF binding proteins to specific MF binding sites (Prestwich et al., 1990; Li and Borst, 1991; Tamone et al., 1997). The binding proteins were identified in the hemolymph, hepatopancreas, and reproductive tissues indicating a reproductive role of MF (Laufer et al., 1996).

As a sesquiterpenoid, MF is structurally homologous to the insect juvenile hormone III (JH III) (Laufer et al., 1987a) and is shown to influence morphological differentiation from adolescent to adult crustaceans (Sagi et al., 1993). The similar biological activity of MF and JH III was first demonstrated by exposing crustacean larvae to JH III. Developmental maturation was interrupted in JH III-exposed crustaceans: exposure inhibited vitellogenesis and spermatogenesis in the mud crab *Rhithropanopeus harrisii*, delayed molt timing in *R. harrisii* (Payen and Costlow, 1977), and induced retention of larval traits in post-larval *H. americanus* (Charmantier et al., 1988). High levels of MF in male *L. emarginata*, induced through eyestalk ablation, prevented allometric growth of their chelae after molting while animals with intact eyestalks successfully molted to a large-claw status (Laufer et al., 2002). Elevated levels of MF in pre-molt crayfish *Procambarus clarkii* led to males molting to the non-reproductive form while low levels of MF prior to molting resulted in crayfish molting to the mature form (Laufer et al., 2005).

Molting and mating are hormonally coordinated (Reddy and Ramamurthi, 1999; Rotllant et al., 2000) and in cold-water crustaceans may be temporally separated (Swiney et al., 2003; Thomton, 2005). *In vitro* studies showed that MF can stimulate organs to secrete ecdysteroids (Tamone and Chang, 1993), and *in vivo* studies linked the

importance of MF and ecdysteroids for males during reproduction (Sagi et al., 1991). Molting physiology can affect reproductive behavior; in *L. emarginata*, new-shell males did not exhibit mating behavior when isolated with a receptive female (Laufer and Ahl, 1995). Molting physiology can also affect reproductive physiology; crustacean gonad development is stimulated by the presence of MF (Nagaraju, 2007), and in new-shell *L. emarginata* reduced reproductive behavior correlated with lower MF levels compared to levels in reproductively active old-shell males (Laufer and Ahl, 1995). High levels of MF in male *L. emarginata* correlated with higher reproductive tissue indices (Sagi et al., 1993; Sagi et al., 1994; Ahl and Laufer, 1996; Ahl et al., 1996; Laufer et al., 1996).

Mating ability may be dependent on time post molt. Sainte-Marie et al. (2008) suggested that male *C. opilio* are sexually inactive directly after molting, an idea affirmed in the wild by observations of mating pairs that did not include any newly molted males (Moriyasu and Comeau, 1996; Sainte-Marie et al., 1999). Both physiological processes are metabolically demanding, with molting requiring, at the least, the synthesis of a new cuticle (Willig and Keller, 1973) and reproduction requiring the synthesis of spermatophores (Sainte-Marie et al., 1995). In the congener, *C. bairdi*, males require at least 99 days post molt before participating in reproduction, regardless of the level of competition for females (Paul et al., 1995). A newly molted male *C. opilio* requires approximately 3 months for carapace hardening from the soft-shell condition (Ernst et al., 2005) and may require significant time after ecdysis to develop or restore gonads sufficient for successful reproduction. This study aims to examine how molting affects

the reproductive physiology of male *C. opilio* by comparing circulating MF and reproductive tissues of adolescents and adults in both the new- and old-shell conditions.

METHODS

Male *C. opilio* were collected from the EBS in the summer of 2008, 2009, and 2010 during the NMFS crab and groundfish trawl surveys (Figure 2). Collections occurred from June 19 to July 1, 2008 (Chilton et al., 2008); from July 11 to July 19, 2009 (Chilton et al., 2009); and from June 30 to July 15, 2010 (Chilton et al., 2011). For each year, near bottom temperatures were calculated by averaging those measured at each site where male *C. opilio* were present during the specified sampling periods (Table 2). For 2008 and 2009, crab were collected at sea and transported live by air from Dutch Harbor to the University of Alaska Southeast in Juneau, AK. In Juneau, crab were held in aquaria with a flow-through seawater system; HOBO® temperature loggers recorded ambient temperatures. Crab were fed an alternating diet of herring, mussels, salmon roe, or squid weekly. Crab collected in 2010 were sampled for hemolymph at sea; whole crabs plus their hemolymph samples were frozen prior to transport to Juneau.

Shell Condition

Shell condition was classified upon arrival in Juneau using the NMFS shell condition index (Jadamec et al., 1999). We retained shell-2, shell-3, and shell-4 crab and divided them into two groups for comparison; "new-shell" crab were those classified as shell-2, having a newly hardened and clean carapace, while "old-shell" crab were a combination

of shell-3 and shell-4 crab, characterized by abrasions and possible epibiotic growth on their carapace. Though soft-shell crab were seen during the summer survey, none were retained for this project.

Terminal Molt and Claw Morphology

Male crab CW and chela height (CH) were measured as described in Jadamec et al. (1999) to the nearest 0.01 mm using digital calipers. The CH and CW were plotted on a logarithmic scale. The discriminant function:

$$\ln(\text{CH in mm}) = -2.8628 + 1.2899*\ln(\text{CW in mm})$$

was used to classify male *C. opilio* as small claw adolescents or large claw adults (Rugolo et al., 2005) (Figure 3). A numerical claw value was calculated for each male:

$$Y_{claw} = 2.8628 + \ln(CH) - 1.2899*\ln(CW)$$

to represent their placement above or below the discriminant function line, and thus represent their maturation status. Males above the line (when Y_{claw} was positive) were taken to be adults and those males below the line (when Y_{claw} was negative) as adolescents. The two groups were fit with linear regressions of ln(CH) versus ln(CW), which were significantly different from one another (ANOVA, $F_{1, 387} = 1595.8$, $p < 0.001$). Over the three-year study period, 41 males were classified as new-shell adolescents, 47 males were classified as old-shell adolescents, 57 males were classified as new-shell adults, and 245 males were classified as old-shell adults.

Methyl Farnesoate Assays

Methyl farnesoate was quantified from hemolymph samples using high performance liquid chromatography (HPLC) (Borst and Tsukimura, 1991). Hemolymph samples (1 mL) were obtained from live crabs using a 1 mL tuberculin syringes fitted with a 25 g needle. Crabs sampled for hemolymph in the laboratory had significantly greater levels of MF than at-sea samples (F-test, $F_{1, 100} = 40.0196$, $p < 0.001$), and because stressed crabs synthesize more MF (Lovett et al., 2001), we chose to only compare samples taken at sea in July 2009 and July 2010. Hemolymph samples were prepared using a triphasic extraction: hemolymph samples were brought to equal volumes using 10% NaCl solution, then extracted with 2500 µL acetonitrile and 500 µL hexane. The hexane phase was removed for HPLC analysis, the acetonitrile fraction was extracted again with 500 µL hexane, and both hexane phases were combined prior to analysis. Hemolymph components were separated through a 250 mm x 4.6 mm silica column. Eluted chemicals were detected at 217 λ and MF was identified using the retention time of MF standards (Echelon, Inc). The total amount of MF was determined by comparing the area under each peak to that of known standards run before each set of samples. The percent recovery of MF after triphasic extraction was determined by extracting known quantities of MF. Percent retention was then calculated as the amount remaining divided by the known original quantity of MF, multiplied by 100%, and was averaged over ten trials. Our extraction efficiency was 87% and this percent retention was applied to all MF results.

Ecdysteroid Assays

Ecdysteroids were measured using a competitive enzyme-linked immunosorbant assay (ELISA) using 20-hydroxyecdysone (20-HE) as the standard (Kingan, 1989; Tamone et al., 2005). Hemolymph samples (1 mL) were obtained from live crabs using a 1 mL tuberculin syringes fitted with a 25 g needle. Crabs were sampled within the first 2 to 3 months of laboratory holding in 2008 and 2009 and at sea in July, 2009. Samples were assayed in duplicate on 96-well plates (Costar). Plates were coated with secondary antibody (Jackson Immunolabs) then blocked with assay buffer containing 0.1% bovine serum albumin (AB/BSA) and 0.002% sodium azide. The plates were washed three times for 5 minutes with phosphate buffered saline containing 0.05% 20-tween (PBST). Standard 20-HE (50 µL) or diluted hemolymph samples (20 µL hemolymph with 30 µL AB/BSA) were added to each well, along with horseradish peroxidase (HRP) conjugated ecdysone (50 µL; 1:4,000) and primary antiserum (50 µL; 1:100,000). The conjugated ecdysone and the primary antiserum were obtained from Dr. Timothy Kingan (U.C. Riverside). Plates were incubated for 24 hours at 4°C. They were washed three times for five minutes with PBST, then developed using a 1:1 solution (100 µL) of TMB peroxidase substrate and peroxidase substrate solution B (KPL, Inc) at room temperature. Development was stopped after 15 minutes with the addition of phosphoric acid (100 µL; 1 M). Absorbances were determined at 450 nm using an ELISA plate reader (BioRad Model 680XR). No results are reported for new-shell males due to low sample size for new-shell male ecdysteroids.

Gonadosomatic Index

Gonad size can be used as a proxy for male *C. opilio* reproductive capacity (Dutil et al., 2009). Crabs were sacrificed by removing the carapace. Reproductive tissues, including testes and vasa deferentia, were dissected from freshly sacrificed crabs, and the gonadosomatic index (GSI) was calculated as a percentage of the wet weight of reproductive tissues (gonad weight, GW) relative to the wet weight of the entire crab. Weights were measured to the nearest 0.01 g. Crabs missing limbs accounted for 48.5% of our samples. We weighed the intact legs on each crab missing limbs to estimate the potential weight of the missing limbs; these weights were added to the total wet weight to account for the loss of mass.

Statistical Analysis

All analyses were conducted using the statistical package R, version 2.12.1 (R Development Core Team, 2005). A p-value of 0.05 was used to reject null hypotheses. Yearly differences were tested between near bottom temperatures and CW using an F-test for each with temperature or CW as the response variable and year as the categorical predictor variable with three levels (2008, 2009, 2010). If there was a significant yearly difference, a post-hoc test of Tukey's Honest Significant Differences (HSD) was used for pairwise comparisons between years.

An analysis of covariance (ANCOVA) with MF as the response variable was used to test differences in male *C. opilio* group (new-shell adolescent, old-shell adolescent, new-shell adult, old-shell adult), year sampled, CW, and interactive terms of male group

with year, male group with CW, and year with CW. We used a fourth-root transformation on MF to satisfy the assumption of normality (Shapiro-Wilks normality test, $p = 0.949$). The post-hoc Tukey's HSD test was run for pairwise comparisons between the four groups of male crabs: new-shell adolescents, old-shell adolescents, new-shell adults, and old-shell adults.

We tested for differences in circulating ecdysteroid levels using an ANCOVA with male *C. opilio* group (old-shell adolescent or old-shell adult), year sampled, CW, and an interactive term of year with CW as variables. We used a power transformation on the response variable, circulating ecdysteroids, to normalize the data (Shapiro-Wilks normality test, $p = 0.403$).

We tested for differences in GSI between male *C. opilio* groups (new-shell adolescent, old-shell adolescent, new-shell adult, old-shell adult), carapace width, year, and the interactive term of carapace width and year using an ANCOVA. Due to high transport mortality in 2008 and 2009, some of the sampled crabs were frozen prior to measuring gonad and whole crab weights, and all crabs sampled in 2010 were frozen; therefore, we included a blocking variable of sample type (fresh or frozen) in the ANCOVA. We natural-log transformed GSI in order to satisfy assumptions of normality (Shapiro-Wilks normality test, $p = 0.376$). Tukey's HSD test was used for pairwise comparisons of GSI among male groups and years sampled. Regressions were performed on ln(GW) and ln(GSI) with respect to ln(CW). An ANOVA was used to test for overall differences in the regressions between the four groups of male *C. opilio*. For both GW and GSI, an ANCOVA was used to test for differences between regression slopes of new-

and old-shell males within adolescent and adult groups, and ANOVA was used to test for differences in intercepts between shell conditions within adolescent and adult groups.

RESULTS

Temperature did not differ across sampling years. Near bottom temperatures were not significantly different within the trawl survey areas where *C. opilio* were present during our 2008, 2009, and 2010 sampling times (F-test, $F_{2, 341} = 0.931$, $p = 0.395$). Variation in CW among years was seen (Table 2): our 2009 survey animals had similar CWs (87.15 ± 3.86 mm) to those from 2010 (86.83 ± 3.12 mm) (Tukey's HSD, $p = 0.991$); however, in 2008 animals (74.46 ± 4.66 mm) were significantly smaller than in both 2009 and 2010 (Tukey's HSD, $p < 0.001$).

Circulating concentrations of MF (Table 3) were significantly different among the four groups of male *C. opilio* (ANCOVA, $F_{3, 65} = 14.542$, $p < 0.001$) (Table 4, Figure 4). The highest concentrations of circulating MF (n = 10, 864.1 ± 290.1 ng/mL) were measured in new-shell adolescent males, which were significantly greater than in old-shell adolescent males (n = 10, 73.0 ± 290.1 ng/mL) and old-shell adult males (n = 43, 257.4 ± 139.9 ng/mL) (Tukey's HSD, $p < 0.001$). Circulating MF increased, albeit with high variability, after the terminal molt when comparing old-shell adolescent males to new-shell adult males (n = 14, 350.1 ± 245.2 ng/mL) (Tukey's HSD, $p = 0.088$). There was no statistically significant difference in MF between new- and old-shell adult males (Tukey's HSD, $p = 0.241$).

Circulating levels of ecdysteroids (Table 3) were not significantly different between old-shell adolescent (n = 13, 4.5 ± 2.0 ng/mL) and old-shell adult (n = 108, 4.8 ± 0.7 ng/mL) male snow crab (ANCOVA, $F_{1, 116} = 0.006$, $p = 0.824$) (Table 5, Figure 5). Ecdysteroids were significantly lower in 2008 (4.1 ± 1.1 ng/mL) compared to 2009 (5.2 ± 0.8 ng/mL) (Table 5).

Average GSI (Table 3) was significantly different among the four groups of male *C. opilio* (ANCOVA, $F_{3, 286} = 70.368$, $p < 0.001$) (Table 6, Figure 6). New-shell adolescent males had a significantly lower GSI (n = 34, 0.47% ± 0.31%) than both old-shell adolescent males (n =27, 0.98% ± 0.35%) and old-shell adult males (n = 180, 1.77% ± 0.13%) (Tukey's HSD, $p < 0.01$). New-shell adult males had significantly lower GSI (n = 55, 0.92% ± 0.24%) than old-shell adult males (Tukey's HSD, $p < 0.01$). However, there was not a statistically significant difference between the GSI of old-shell adolescent and new-shell adult male *C. opilio* (Tukey's HSD, $p = 0.997$). There was no significant difference between GSI calculated from freshly sampled crabs and those that had been frozen prior to sampling (ANCOVA, $F_{1, 286} = 1.118$, $p = 0.291$) (Table 6). Freezing crabs before transporting, thawing, and dissecting their testes and vasa deferentia enabled us to remove the problem of transport mortality on live crabs.

There was annual variation in GSI (ANCOVA, $F_{2, 286} = 21.014$, $p < 0.001$) (Table 6). Average GSI was significantly lower in 2009 (0.94% ± 0.18%) compared to 2008 (2.38% ± 0.22%) (Tukey's HSD, $p < 0.001$) and 2010 (1.24% ± 0.15%) (Tukey's HSD, $p < 0.05$), and was significantly lower in 2010 compared to 2008 (Tukey's HSD, p <

0.001). Collection location varied by year, and crabs collected in 2009 were sampled from more northwesterly sites than those from 2008 and 2010 (Figure 2).

For all groups, GW increased with CW (Figure 7). The relationship of ln(GW) to ln(CW) was significantly different between new- and old-shell males (ANOVA, $F_{1, 292} = 59.041$, $p < 0.001$) and between adolescent and adult males (ANOVA, $F_{1, 292} = 120.012$, $p < 0.001$). New-shell adolescent males exhibited the strongest relationship between ln(GW) and ln(CW) (adjusted $R^2 = 0.39$) whereas old-shell adolescent males, new-shell adult males, and old-shell adult males had weaker relationships (adjusted $R^2 = 0.31$, 0.29, and 0.26, respectively) (Table 5). The GSI of male *C. opilio* decreased with increasing CW (ANCOVA, $F_{1, 286} = 17.774$, $p < 0.001$) (Table 6, Figure 8). However, the relationship was statistically significant only for old-shell adult males (ANOVA, $F_{1, 156} = 41.95$, $p < 0.001$) (Table 7).

DISCUSSION

This project examined two aspects of EBS *C. opilio* reproduction, hormones and reproductive tissues, to investigate the effect of molting physiology on reproductive physiology between adolescent and adult males. We hypothesized that MF, as a reproductive hormone, would circulate at higher concentrations in adult males when compared to adolescent males. However, our results were contrary to our hypothesis; the mean MF levels for adult males were lower than for new-shell adolescent male MF. Nevertheless, these results support a role for MF in the morphological differentiation that occurs after the terminal molt. The decrease of MF from new-shell adolescents to old-

shell adolescents may indicate the role of MF in the regulation of allometric growth for *C. opilio* as seen in other decapod crustaceans (Laufer et al., 2002; 2005). Old-shell adolescent males may require lower circulating MF to effectively molt to morphometric maturity rather than molt and remain in the adolescent phase.

Comparably lower levels of MF in a given life history stage indicate smaller reproductive structures for several crustaceans. For example, levels of circulating MF in *L. emarginata* mimicked the variation of reproductive system indices when comparing new- and old-shell adolescent and adult males (Sagi et al., 1994; Laufer and Ahl, 1995; Ahl and Laufer, 1996; Laufer et al., 1996). The snow crab congener, *C. bairdi*, had both low GSI and MF in new-shell males compared to old-shell males (Laufer et al., 1996). However, high levels of circulating MF in *C.opilio* did not coincide with high values of GSI in our study.

New-shell adult male *C. opilio* had lower GSI than old-shell adult males, suggesting the necessity for time after the terminal molt to reach the full potential of gonad development. The pattern of reduced gonads in new-shell adult males compared to old-shell adult males was also seen in eastern Canadian *C. opilio* taken in the Gulf of Saint Lawrence (Sainte-Marie et al., 1995). Both growth and gonad development are energetically demanding, and either process may compromise the other. Growth-per-molt (molt increment) decreases in adolescent males compared to juvenile males hypothetically due to the onset of spermatophore production (Miller and Watson, 1976; Sainte-Marie et al., 1995; Comeau et al., 1998), therefore greater energy is available for gonad development once growth ceases.

Whereas gonad development is stimulated by MF in several crustaceans, other terpenoids may regulate crustacean reproduction in place of MF (Tobe et al., 1989; Mak et al., 2005). Farnesoic acid (FA) is a sesquiterpenoid and the precursor of MF (Tobe et al., 1989; Borst et al., 2001); synthesis of MF from FA is catalyzed by farnesoic acid O-methyl transferase (Wainwright et al., 1998) and the presence of excess FA stimulates MF production (Kwok et al., 2005). However, FA may stimulate gonad development more directly as demonstrated with the more effective and consistent induction of vitellogenesis by FA than MF in the red crab *Charybdis feriatus* (Chan et al., 2005). While old-shell males had greater GSI, they did not have significantly higher levels of circulating MF in our study animals; nevertheless perhaps male *C. opilio* gonad production is regulated by other crustacean hormones.

Ecdysteroids may be important endocrine regulators for gonad development. Addition of ecdysteroids affected testicular cellular production of *H. americanus* (Brody and Chang, 1989) and the freshwater prawn *Macrobrachium rosenbergii* (Sagi et al., 1991). Additionally, 20-hydroxyecdysone stimulated vitellogenin production in lobster *H. americanus* hepatopancreas tissue (Tiu et al., 2009; Tiu et al., 2010). The presence of ecdysteroids in the testes of terminally molted male *L. emarginata* suggests an active function of ecdysteroids in reproduction (Rotllant et al., 2000). Ecdysteroids measured in adolescent and adult intermolt *C. opilio* were not significantly different (Figure 5), and were comparable to concentrations measured previously in adult male *C. opilio* (3.98 ± 0.8 ng/mL) (Tamone et al., 2005). While the adult males no longer require ecdysteroids for ecdysis, they may maintain circulating ecdysteroids for spermatogenesis.

Of our study animals, old-shell adult male *C. opilio* may have been the most reproductively active prior to being sampled. The significant inverse relationship of GSI to CW (Figure 6) may indicate that larger old-shell adult males are copulating. Reduced GSI in larger old-shell adult males due to mating activity may also explain the shallower pattern of ln(GW) observed across ln(CW) when compared to the other male groups (Table 7, Figure 8); lighter reproductive structures than those expected for their size results in a smaller regression slope when modeling the GW and CW relationship. No other male groups had a significant relationship between GSI and CW (Table 7); therefore, large adolescent and new-shell adult males may not have been using their reproductive stores.

Male *C. opilio* from the EBS in our study had similar GSI to CW relationships to those from Atlantic Canada. Vasosomatic index (the ratio of only vas deferens to whole crab wet weight) was inversely correlated with CW for adult *C. opilio* from the Gulf of Saint Lawrence, and in adolescent males displayed no significant relationship with CW (Comeau and Conan, 1992). Despite the differences in the measurement of reproductive tissues, we also found inverse relationships between the reproductive index, GSI, and CW (Figure 7); these relationships were significant only in old-shell adult males (Table 5), though the other groups had low sample sizes.

While EBS and Gulf of Saint Lawrence *C. opilio* have similar GSI relationships across CW, EBS males may have lower GW compared to Canadian males. Average adjusted ln(GW) in intermediate and old-shell adults from Baie Sainte-Marguerite were 1.246 ± 0.025 and 1.535 ± 0.026, respectively(Sainte-Marie et al., 1995), while new- and

old-shell adult adjusted ln(GW) from our study were 0.84 ± 0.15and 1.08 ± 0.09 (Figure 8B). Lower GW in adult EBS male snow crab compared to Canadian male snow crab GW may suggest lower sperm availability or allotment for EBS female snow crabs; male reproductive output inferred from spermathecal loads in females was smaller in the EBS females compared to females sampled from the Gulf of Saint Lawrence (Slater et al., 2010). As such, EBS females may experience sperm limitation in fertilizing second clutches if their needs are similar to Canadian female snow crabs.

Differences in male reproductive capacity were seen not only between oceans but also within the EBS. The significant difference of GSIs between years of our study may be attributed to either sampling in different areas within the eastern Bering Sea trawl survey (Figure 2) or different near bottom temperatures during the surveys. Males sampled from 2009 had the lowest average GSI and were sampled from the most northwesterly latitudes of the survey. While average size of snow crab decreases with increasing latitude (Otto, 1998; Zheng et al., 2001; Burmeister and Sainte-Marie, 2010) due to the physiological constraints of colder temperatures on *C. opilio* (Foyle et al., 1989), our animals were not found in significantly different temperatures. Crab from 2008 had significantly smaller CWs than crab from 2009/2010, but the mechanism behind the size difference does not appear to be temperature related. Temperature may explain yearly differences in male GSI indirectly through female clutch retention. The low temperature in 2008 (Table 2) may have led to female *C. opilio* maintaining a biennial cycle (Moriyasu and Lanteigne, 1998). If females were on a biennial cycle in 2008, they would be less available for mating that year but receptive to males the

following year, thus potentially explaining lower sperm reserves in 2009 compared to 2008 from higher reproduction prior to sampling in 2009.

Molt timing may preclude newly molted males reproducing with females. Female *C. opilio* in the EBS undergo their terminal molt in the winter (Ernst et al., 2005), whereas males undergo their terminal molt in the late spring (Rugolo et al., 2005). Newly molted adult males may not be able to mate with females until the following year due to their reduced reproductive capacity, as well as temporal differences between males and receptive females. Further, the current EBS *C. opilio* fishery begins in October and runs through May, with peak harvests occurring in January (Bowers et al., 2011). If new-shell males are harvested in the first year following their terminal molt, they may not have the opportunity to mate before being caught.

Knowledge of male reproduction and contribution to the stock is important in maintaining the health of the EBS *C. opilio* fishery. While the current stock assessment considers all terminally molted male snow crab reproductively active, there appears to be a reproductive diapause in newly molted, hard shell males, evidenced by lower GSI compared to old-shell males. The timing of male molting and recovery from molting in conjunction with the timing of the fishery and female availability may therefore inhibit new-shell males from mating before being harvested. More research is needed to understand the complex hormonal regulation of male *C. opilio* development and reproduction.

ACKNOWLEDGEMENTS

We would like to thank our funding, North Pacific Research Board (Project 812). We are grateful for the help of NMFS and ADF&G with crab collections. We also thank the University of Alaska Southeast and the Alaska Fisheries Science Center's Auke Bay Marine Station for the facilities to house our snow crabs, the Atkinson lab for sharing space for the HPLC, and UAS/UAF students for their help with crab husbandry.

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TABLES

Table 1. Decapod crustaceans in which methyl farnesoate has been identified.

Animal	Species	Common Name	Source
Crab	*Libinia emarginata*	Spider crab	Laufer et al., 1987a, 1987b; Borst et al., 1987
	Cancer borealis	Rock crab	Borst et al., 1987
	Cancer pagurus	Edible crab	Wainwright et al., 1996a, 1996b
	Callinectes sapidus	Blue crab	Henry and Borst, 2006
	Carcinus maenas	Green crab	Borst et al., 1987
	Charybdis feriatus	Red crab	Mak et al., 2005
	Chionoecetes bairdi	Tanner crab	Laufer et al., 1996
	Chionoecetes opilio	Snow crab	Mitchell, 2004
	Libinia dubia	Longnosed spider crab	Laufer and Biggers, 2001
	Metacarcinus magister [#]	Dungeness crab	Tamone and Chang, 1993
	Ovalipes ocellatus	Lady crab	Laufer and Biggers, 2001
	Oziotelphusa senex senex	Indian field crab	Kalavathy et al., 1999
	Scylla serrata	Mud crab	Tobe et al., 1989
	Uca pugnax	Atlantic marsh fiddler crab	Laufer and Biggers, 2001
	Uca triangularies	Fiddler crab	Nagaraju, 2007
Lobster	*Homarus americanus*	American lobster	Borst et al., 1987
	Homarus gamarus	European lobster	Laufer and Biggers, 2001
	Nephrops norvegicus	Norway lobster	Rotllant et al., 2001
Crayfish	*Cambarus bartonii*	Common crayfish	Borst et al., 1987
	Cherax quadricarinatus	Redclaw crayfish	Soroka et al., 2000
	Procambarus clarkii	Red swamp crayfish	Laufer et al., 1998
Prawn/	*Fenneropenaeus chinensis**	Chinese white shrimp	Laufer and Biggers, 2001
Shrimp	*Farfantepenaeus duorarum**	Pink shrimp	Laufer and Landau, 1991
	*Litopenaeus setiferus**	Northern white shrimp	Laufer and Biggers, 2001
	*Litopenaeus stylirostris**	Western blue shrimp	Laufer and Biggers, 2001
	*Litopenaeus vannamei**	Whiteleg shrimp	Laufer and Landau, 1991
	Macrobrachium malcomsonii	Monsoon river prawn	Nagaraju et al., 2003
	Macrobrachium rosenbergii	Freshwater prawn	Sagi et al., 1991
	Penaeus monodon	Giant tiger prawn	Laufer and Biggers, 2001
	Penaeus semisulculatus	Green tiger prawn	Laufer and Landau, 1991
	Sicyonia ingentis	Ridgeback shrimp	Chang et al., 1992

[#]formerly genus *Cancer*, *formerly genus *Penaeus*

Table 2. Average near bottom temperature (NBT) of sites sampled in 2008, 2009, and 2010 where male *C. opilio* were present and average carapace width (CW) of male *C. opilio* sampled in each year.

Year	NBT ± 95% CI (°C)	CW ± 95% CI (mm)
2008	0.72 ± 0.27	74.46 ± 4.66
2009	0.82 ± 0.39	87.15 ± 3.86
2010	1.00 ± 0.30	86.83 ± 3.12

Table 3. Average gonadosomatic index (%), circulating methyl farnesoate (ng/mL), and circulating ecdysteroids in eastern Bering Sea male *C. opilio*.

	Gonadosomatic index		Circulating methyl farnesoate		Circulating ecdysteroids	
Male *C. opilio* Group	n	Average ± 95% CI (%)	n	Average ± 95% CI (ng/mL)	n	Average ± 95% CI (ng/mL)
New-shell Adolescent	34	0.47 ± 0.31	10	864.1± 290.1		
Old-shell Adolescent	27	0.98 ± 0.35	10	73.0± 290.1	13	4.5±2.0
New-shell Adult	55	0.92 ± 0.24	14	350.1± 245.2		
Old-shell Adult	180	1.77 ± 0.13	43	257.4± 139.9	108	4.8± 0.7

Table 4. Results from the ANCOVA for the effects on circulating methyl farnesoate of male *C. opilio* group (new-shell adolescent, old-shell adolescent, new-shell adult, old-shell adult), year sampled (2009 or 2010), carapace width (mm), the interaction between shell condition and year, and the interaction between carapace width and year. Statistics: *d.f.*, degrees of freedom; MS, mean squares; F, variance ratio; p-value.

Variable:	*d.f.*	MS	F	p-value
male group	3	14.54	9.286	< 0.001
year	1	27.68	17.675	< 0.001
carapace width	1	1.82	1.136	0.285
male group * year	2	7.43	4.747	0.012
male group * carapace width	3	6.70	4.280	0.008
year * carapace width	1	6.37	4.066	0.048
error	65	1.57		

Table 5. Results from the ANCOVA for the effects on circulating ecdysteroids of male *C. opilio* group (old-shell adolescent, old-shell adult), year sampled (2008 or 2009), carapace width (mm), and the interaction between carapace width and year. Statistics: *d.f.*, degrees of freedom; MS, mean squares; F, variance ratio; p-value.

Variable:	*d.f.*	MS	F	p-value
male group	1	0.01	0.050	0.824
year	1	0.69	5.633	0.019
carapace width	1	2.74	22.375	< 0.001
year * carapace width	1	0.48	3.949	0.049
error	116	0.12		

Table 6. Results from the ANCOVA for the effects on gonadosomatic index of male *C. opilio* group (new-shell adolescent, old-shell adolescent, new-shell adult, old-shell adult), year sampled (2008, 2009, 2010), carapace width (mm), the interaction between carapace width and year, and sample type (fresh, frozen). Statistics: *d.f.*, degrees of freedom; MS, mean squares; F, variance ratio; p-value.

Variable:	*d.f.*	MS	F	p-value
male group	3	22.02	70.368	< 0.001
year	2	6.58	21.014	< 0.001
carapace width	1	17.77	56.795	< 0.001
sample type	1	0.35	1.118	0.291
year * carapace width	2	3.26	10.402	< 0.001
error	286	0.31		

Table 7. Relationship between gonad weight (GW) and gonadosomatic index (GSI) as a function of carapace width (CW) in male *C. opilio* collected in the eastern Bering Sea in July 2008, 2009, and 2010. Ranking of intercepts are shown.

Group	ln(GW) = a + b*ln(CW)					ln(GSI) = a + b*ln(CW)				
	a	b	adj R^2	F	p-value	a	b	adj R^2	F	p-value
(1) New-shell Adolescent	-11.32	2.65	0.39	8.707	0.016	0.20	-0.13	-0.09	0.02697	0.872
(2) Old-shell Adolescent	-9.11	2.29	0.31	22.49	< 0.001	3.81	-0.82	0.04	3.067	0.087
Slopes: (1) = (2)				0.6011	0.441				0.7067	0.404
Intercepts: (1) < (2)				16.425	<0.001				13.8058	< 0.001
(3) New-shell Adult	-10.56	2.42	0.29	31.3	< 0.001	1.38	-0.44	0.003	1.265	0.264
(4) Old-shell Adult	-6.34	1.68	0.26	56.21	< 0.001	6.44	-1.41	0.21	41.95	< 0.001
Slopes: (3) = (4)				0.053	0.818				0.3242	0.570
Intercepts: (3) < (4)				38.928	<0.001				26.259	< 0.001

FIGURES

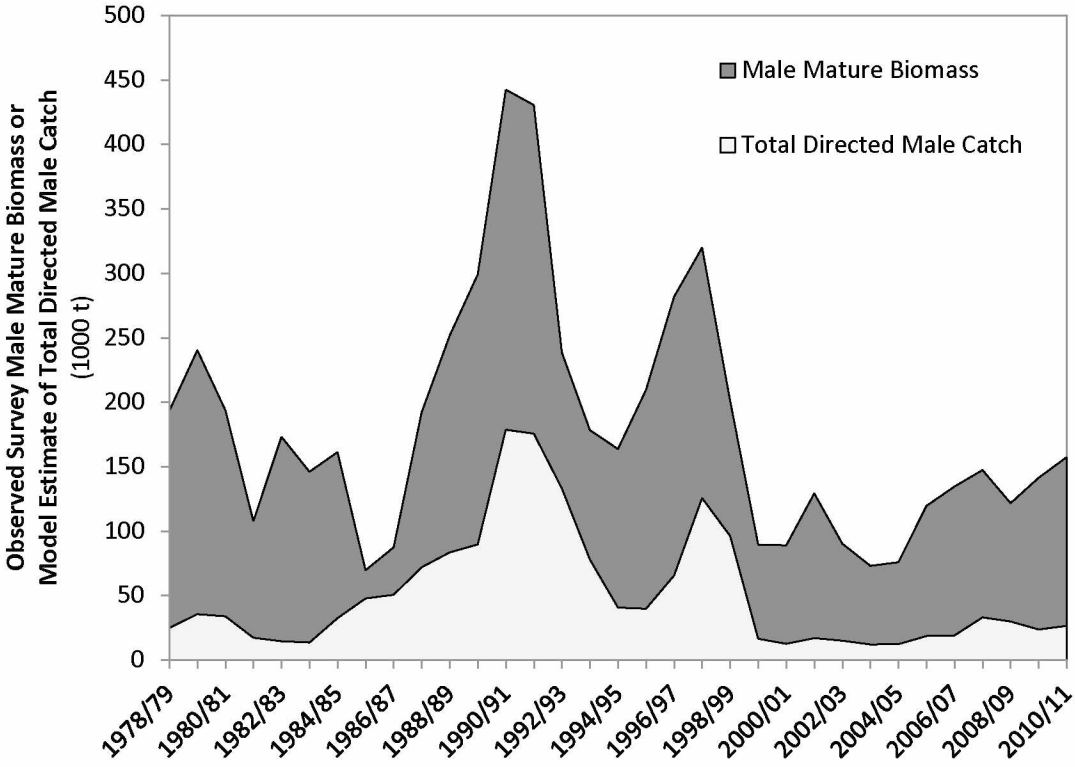

Figure 1. Observed survey male mature biomass and estimated total directed male catch in 1000 t of eastern Bering Sea snow crab from the 1978/1979 season to the 2010/2011 season, adapted from NPFMC (2011).

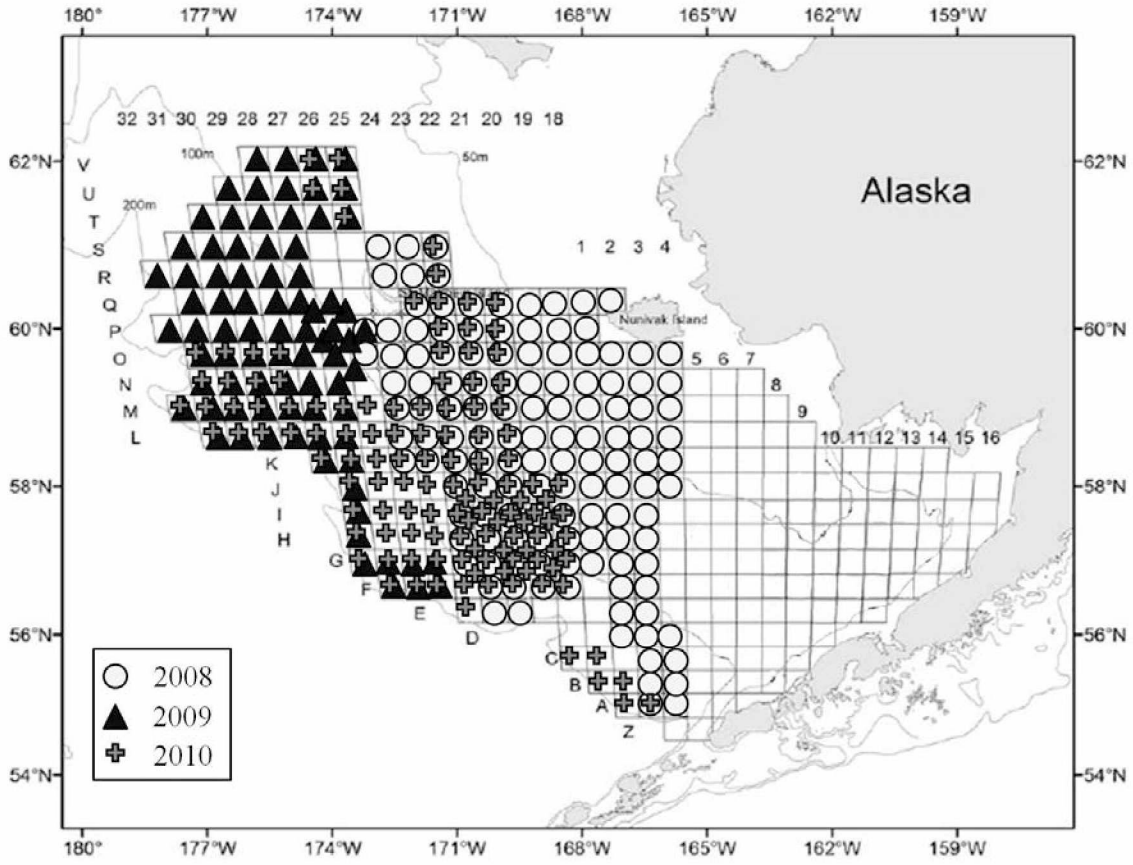

Figure 2. Areas sampled for snow crab during the eastern Bering Sea trawl survey in 2008 (○, June 19 to July 1), 2009 (▲, July 11 to July 19), and 2010 (grey ✚, June 30 to July 15). Figure adapted from Chilton et al. (2011).

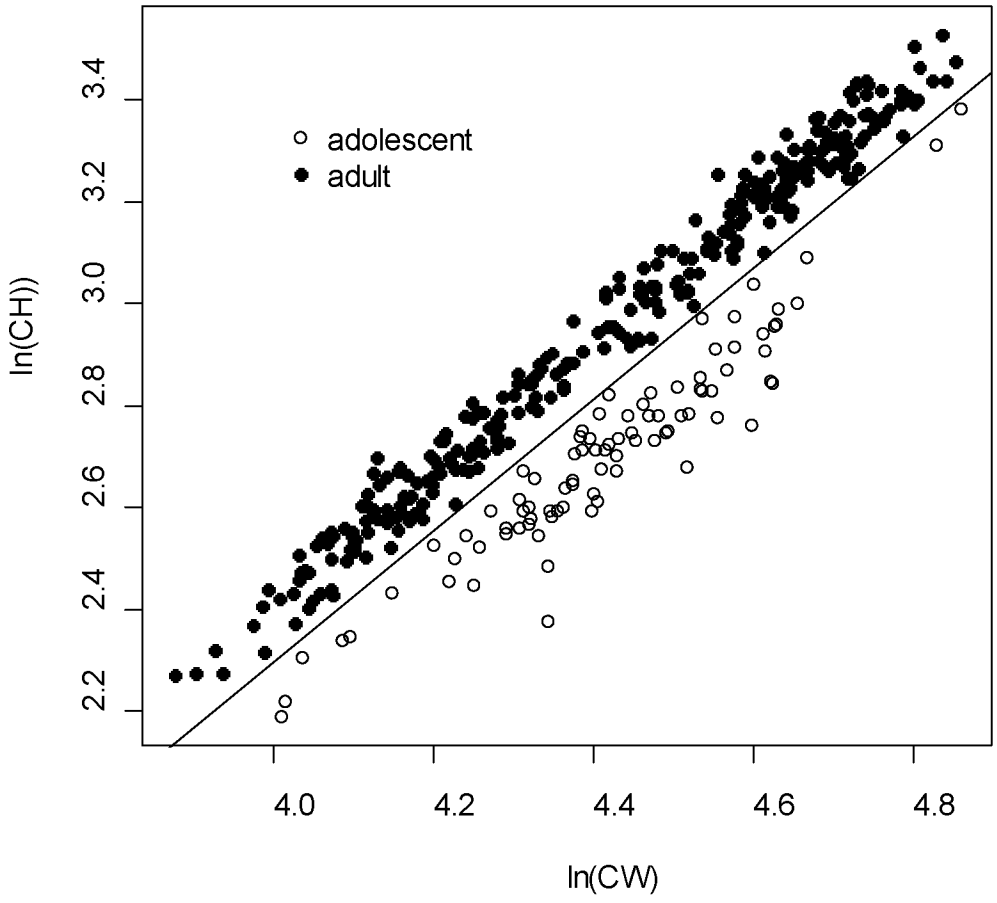

Figure 3. Chela allometry for eastern Bering Sea male snow crab distinguished by a logarithmic disciminant function (solid line, Rugolo et al., 2005): ln(chela height (CH) in mm) = -2.8628 + 1.2899*ln(carapace width (CW) in mm). Open circles (○) represent small-claw adolescent males (n = 88) with a fit line of ln(CH) = -2.6410 + 1.21096*ln(CW). Closed circles (●) represent large-claw adult males (n = 302) with a fit line of ln(CH) = -2.7611 + 1.2933*ln(CW). The groups of adolescent and adult males were significantly different from one another (ANOVA, $F_{1, 387} = 1595.8$, $p < 0.001$).

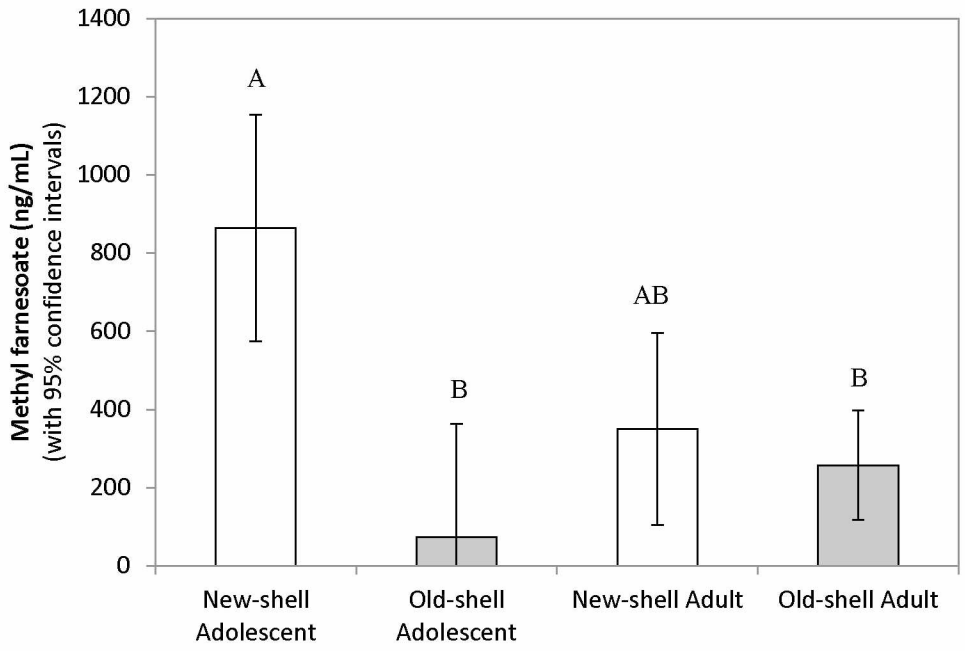

Figure 4. Mean circulating levels of methyl farnesoate (MF, ng/mL) in male *C. opilio* with 95% confidence intervals. Circulating MF was significantly greater in new-shell adolescents (n = 10) than old-shell adolescents (n = 10) and old-shell adults (n = 43) (Tukey's HSD, $p < 0.001$), but not significantly different in new-shell adults (n = 14) (Tukey's HSD, $p = 0.210$). Letters above the bars represent significantly different groups.

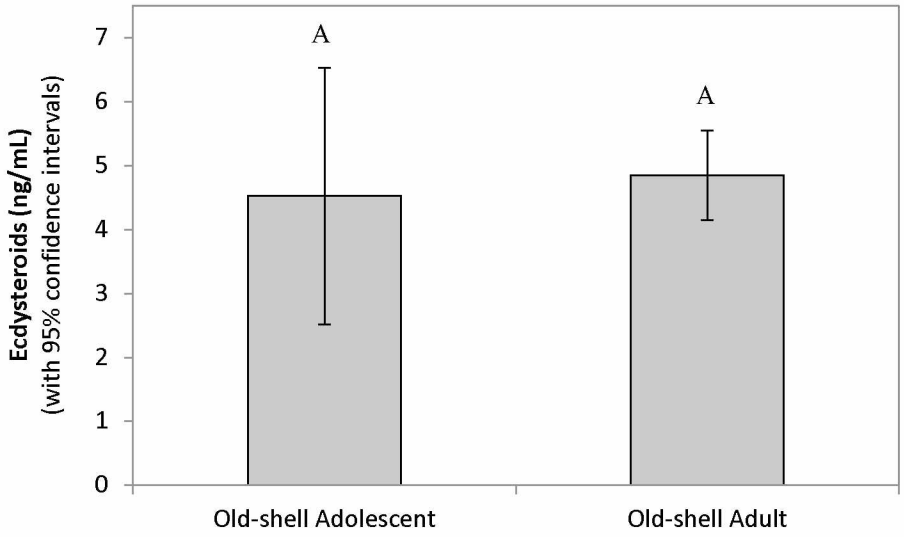

Figure 5. Mean circulating levels of 20-hydroxyecdysone (20-HE, ng/mL) in male *C. opilio* with 95% confidence intervals. Circulating 20-HE was measured in old-shell adolescents (n = 13) and old-shell adults (n = 108). There were no significant differences of circulating 20-HE between groups (ANCOVA, F = 0.0003, p = 0.986).

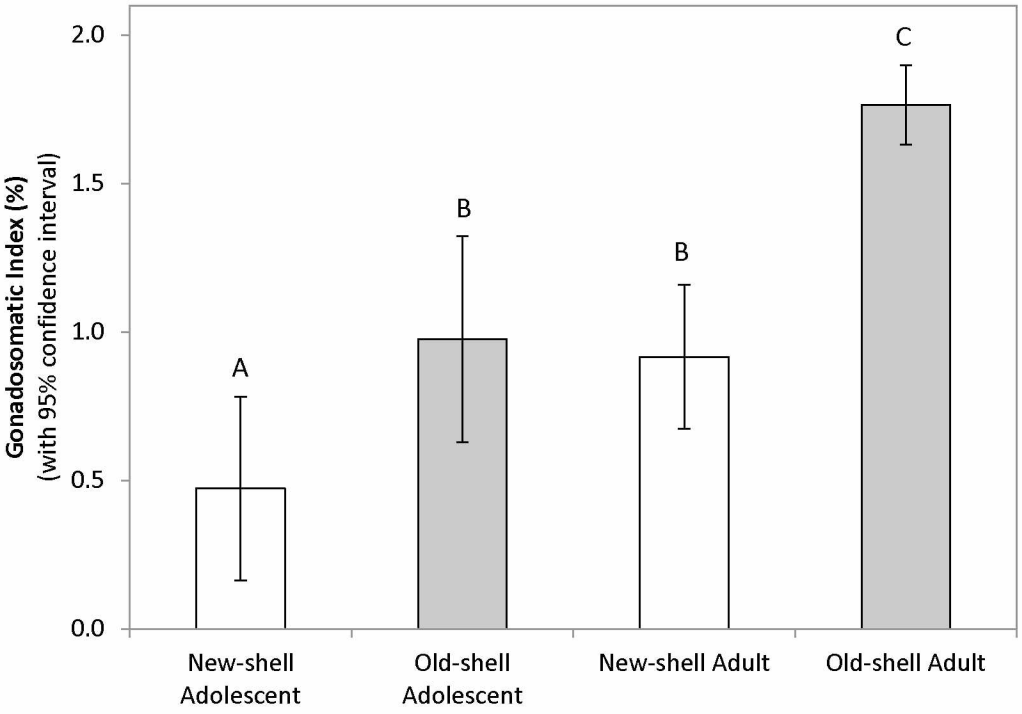

Figure 6. Mean gonadosomatic index (GSI, %) with 95% confidence intervals for shell condition in both adolescent and adult male snow crab. Letters above the bars represent significantly different groups. GSI was lower in new-shell males compared to old-shell males, in both adolescents and adults (ANCOVA, $F_{1, 288} = 149.273$, $p < 0.001$). New-shell adolescent male GSI (n = 34) was lowest and old-shell adult male GSI (n = 180) was highest compared to all other maturity and shell types (Tukey's HSD, $p < 0.01$). Old-shell adolescent (n = 27) male GSI was not significantly different from new-shell adult (n = 55) male GSI (Tukey's HSD, $p = 0.9971$).

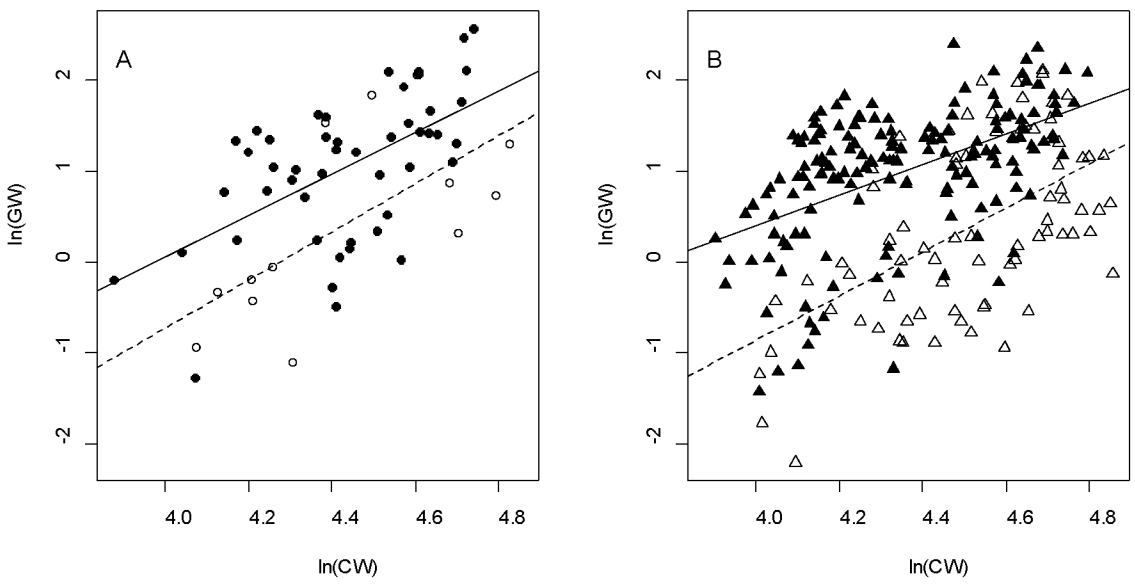

Figure 7.Gonad weight (GW, g) increased with carapace width (CW, mm) on a natural logarithmic scale for (A) adolescent and (B) adult male snow crab. Open circles (○) represent new-shell adolescent males (n = 34) and closed circles (●) represent old-shell adolescent males (n = 27). Open triangles (△) represent new-shell adult males (n = 55) and closed triangles (▲) represent old-shell adult males (n = 180). The dashed line in 5A is the linear regression for new-shell adolescent males (slope = 2.65, adjusted $R^2 = 0.39$, $p = 0.013$) and the solid line in 5A is the linear regression of old-shell adolescent males (slope = 2.29, adjusted $R^2 = 0.31$, $p < 0.01$). The dashed line in 5B is the linear regression for new-shell adult males (slope = 2.42, adjusted $R^2 = 0.29$, $p < 0.01$) and the solid line in 5B is the linear regression of old-shell adult males (slope = 1.68, adjusted $R^2 = 0.26$, $p < 0.01$).

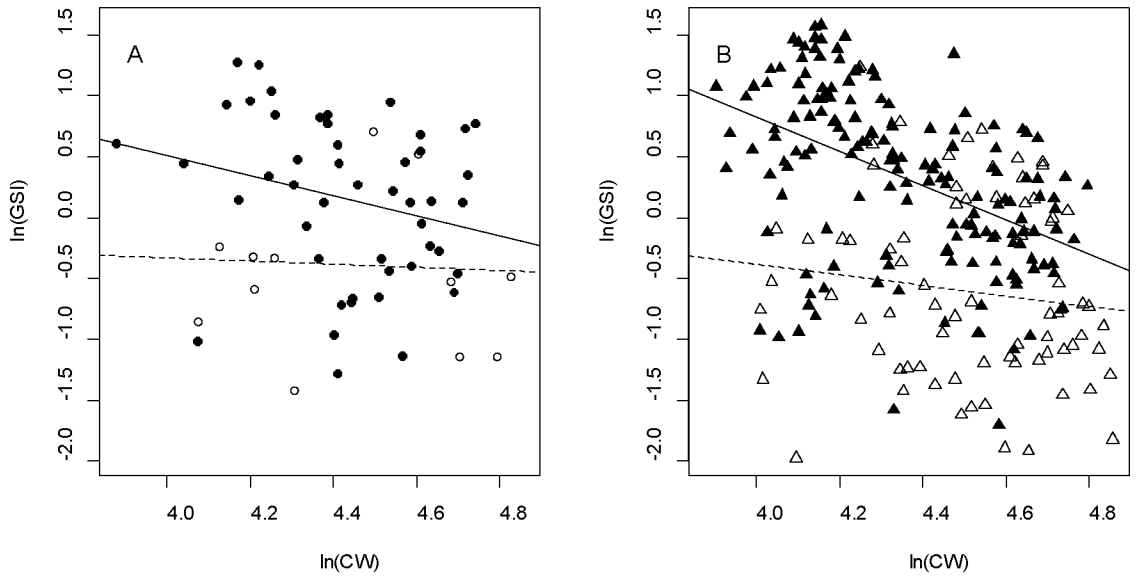

Figure 8.Gonadosomatic index (GSI, %) had an inverse relationship with carapace width (CW, mm) on a natural logarithmic scale for (A) adolescent and (B) adult male snow crab. Open circles (○) represent new-shell adolescent males (n = 34) and closed circles (●) represent old-shell adolescent males (n = 27). Open triangles (△) represent new-shell adult males (n = 55) and closed triangles (▲) represent old-shell adult males (n = 180).The dashed line in 6A is the linear regression for new-shell adolescent males (slope = -0.13, adjusted R^2 = -0.09, p = 0.87) and the solid line in 6A is the linear regression of old-shell adolescent males (slope = -0.82, adjusted R^2 = 0.04, p = 0.09). The dashed line in 6B is the linear regression for new-shell adolescent males (slope = -0.44, adjusted R^2 = 0.003, p = 0.26) and the solid line in 6B is the linear regression of old-shell adolescent males (slope = -1.41, adjusted R^2 = 0.21, $p < 0.01$).

General Conclusion

Male reproductive physiology has a complex relationship with molting physiology. Our study suggests MF is more important as a juvenile hormone rather than a gonadotropic hormone in *C. opilio*. Unlike the congener *C. bairdi*, in which new-shell males had both lower MF levels and reproductive indices than old-shell males (Laufer et al., 1996), our *C. opilio* males had a significant decrease in MF prior to the terminal molt (Figure 4), despite old-shell adolescent males having significantly higher GSI compared to new-shell adolescents (Figure 6). More research regarding the roles of MF, its precursor farnesoic acid, and ecdysteroids for male reproduction and gonad development is needed to advance our understanding of the molting cycle's effect on male participation in reproducing.

Results of this study indicate *C. opilio* males compromise their gonad development during the energetically expensive molting process. The knowledge that newly molted adult males are not as reproductively fit as older males should be included when making stock assessments. While new-shell make up a high proportion of the EBS catch (Bowers et al., 2011), other fisheries do not place as much emphasis on the hard clean appearance of snow crab. New-shell crab are not targeted in the Gulf of Saint Lawrence fishery, which occurs mostly in the late spring and early summer; recently molted crab made up only 11.5% of the 2011 season catches in an area only fished after June 30 (DFO, 2012).

Management of the EBS *C. opilio* stock regards mature male biomass as equivalent to the biomass of adult males (NPFMC, 2011), but due to size limits based on

carapace width alone, a proportion of the harvested stock is still adolescent males. Using our measured crabs, 17.6% of males > 101 mm CW were adolescents. One possible management change would be to implement both a CW and CH size limit in an attempt to harvest only adult males and ultimately maximize yield per recruit by allowing unfished adolescent males to terminally molt. Removing adolescent males from the catch may enable them to contribute both genetically to the stock and volumetrically to the catch after growing through their terminal molt (Comeau and Conan, 1992; Sainte-Marie et al., 1995).Variability in stock recruitment and the newly recovered status of EBS *C. opilio* has motivated a greater focus on EBS snow crab research. Combining the knowledge of lower GSI in new-shell males compared to old-shell males with size limit management may further the stock's recovery.

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